May 4, 2012

Dr. Hardy Edwards III
Vitamin Derivatives, Inc.
625 Lem Edwards Road
Winterville, Georgia 30683

Re: GRAS Notice No. AGRN 000-009

Dear Dr. Edwards:

The Food and Drug Administration (FDA) is responding to the notice, dated June 14, 2011 that you submitted, on behalf of Vitamin Derivatives, Inc. (VDI) under FDA's Center for Veterinary Medicine (CVM) Pilot Program for substances generally recognized as safe (GRAS) added to food for animals (See 75 FR 31800; June 4, 2010). FDA's Center for Veterinary Medicine received the notice on June 17, 2011, filed it on July 13, 2011, and designated it as GRAS Notice No. AGRN 000-009.

The subject of your notice is 1-alpha-hydroxycholecalciferol. The notice informs FDA of the view of VDI, that 1-alpha-hydroxycholecalciferol is GRAS, through scientific procedures, for use as an ingredient in broiler feed as a source of vitamin D in broilers up to 5 μg/kg in the finished feed.

FDA has evaluated the information that VDI discusses in its GRAS notice as well as other data and information that are available to the agency. As discussed more fully below, the notice does not provide a sufficient basis for a determination that 1-alpha-hydroxycholecalciferol is GRAS under the conditions of its intended use in animal food.

Data and information that VDI presents to support its GRAS determination

VDI describes the common name of the ingredient, conditions of use, specifications and analytical methods, analysis of lots, physical description, and method of manufacture. Public information included general manufacturing methods.

VDI includes an opinion letter from a GRAS panel they convened to evaluate the safe use of 1-alpha-hydroxycholecalciferol as a source of vitamin D in broilers. The panel members conclude in a signed statement, that 1-alpha-hydroxycholecalciferol is GRAS through scientific procedures for use as a source of vitamin D for broilers at levels up to 5 μg/kg feed.

VDI provides literature references to support the use of 1-alpha-hydroxycholecalciferol as a vitamin D_3 derivative that has been shown to exhibit vitamin D activity in broilers. VDI includes published reference articles that discuss quantitative evaluation of 1-alpha-hydroxycholecalciferol as a cholecalciferol substitute for broilers.

To address human food safety and toxicity, the GRAS monograph provided a summary of the published literature on the metabolism, acute and subacute toxicity, developmental and reproductive toxicity, teratogenicity and mutagenicity of 1-alpha-hydroxycholecalciferol. The

notice also addressed its uses in human medicine for the treatment of renal osteodystrophy, hyperparathyroidism, osteoporosis and other disorders, and the dosage levels found safe in humans for its medicinal use. The notice concluded that the thirty-year record of use, with a minimum of serious side effects, argues strongly that the recommended small doses are safe in humans. The notice indicated that the danger of taking too much vitamin D in humans is hypercalcemia and the potential teratogenic effect. To assess the human exposure, the GRAS monograph estimated a daily human intake of 1-alpha-hydroxycholecalciferol from consumed poultry products based on available literature and several unreferenced assumptions.

To address target animal safety, VDI referenced a 42-day broiler safety study published by Pesti and Shivaprasad (2010) as pivotal data to support the safety of 1-alpha-hydroxycholecalciferol for the target animal species. VDI also included information on metabolism, acute and subacute toxicity, developmental and reproductive toxicology, mutagenicity, and carcinogenicity.

FDA's evaluation of the data and information in VDI's notice

FDA has the following comments regarding manufacturing chemistry:

1. The notice does not adequately address raw ingredient specifications for the starting material, vitamin D_3, or the ingredients that make up the final pre-mix, including: starch, peanut oil, sorbitan monostearate, FD&C Green #3, sorbic acid, butylated hydroxytoluene (BHT), sodium benzoate, ground limestone, ground rice hulls, and mineral oil.

2. We question if all of the ingredients in the final pre-mix are being used at levels approved for animal feed. BHT is listed as a GRAS affirmed substance in 21 CFR 582.3173 "when the total content of antioxidants is not over 0.02 percent of fat or oil content, including essential (volatile) oil content of food provided the substance is used in accordance with good manufacturing or feeding practice." The notice did not provide enough information to ensure that the BHT in the final pre-mix meets this requirement.

3. The notice did not address the known impurities in 1-alpha-hydroxycholecalciferol and include these in the ingredient specifications for the pure compound.

4. The method included in the notice to quantify pure 1-alpha-hydroxycholecalciferol is not described in sufficient detail to understand how the method is executed. The AOAC method referenced in the notice appears to be sufficiently different than the method included such that the notice should include a method validation summary. The procedure does not include any information on method calibration, limit of detection, or limit of quantification, nor did the method address any impurities present.

5. A method to extract and quantify the amount of 1-alpha-hydroxycholecalciferol in the final pre-mix should be included in the notice. Since the substance is a form of vitamin D, the notice should demonstrate that the content in the final pre-mix is as it was intended.

6. The stability of vitamins and vitamin sources is an important aspect of the manufacture of animal feeds because of their importance to animal nutrition. The notice did not include sufficient information about the stability of the substance from publically available sources, which can be corroborated by unpublished data. The information that was included in the notice was from an informal study in an uncontrolled environment, which is not sufficient to demonstrate stability. Publically available information to demonstrate the stability of 1-alpha-hydroxycholecalciferol for the intended shelf life was not in the notice. Supporting unpublished material, including analytical methodology and sample analysis from 3-5 lots could be used to demonstrate the stability of the ingredient throughout the intended shelf-life. Since the packaging and storage are critical to the stability, information on the identity of the packaging materials is important. Also, the notice did not address the stability of the pre-mix through drum-drying and pelleting.

7. Homogeniety is an important aspect of animal feed. The notice does not address the homogeneous distribution of 1-alpha-hydroxycholecalciferol in the pre-mix and final feed matrix for the intended animal species supported by publically available sources.

FDA has the following comments regarding human food safety:

8. The notice discussed the results of extensive oral toxicological studies in animals and identified the two main concerns associated with excess intake (hypercalcemia and teratogenic effects). We have some questions regarding these animal test results: (1) the notice lacks a comprehensive discussion of the key references; for example, no-observed-effect-levels (NOELs) from a series of repeat-dose oral toxicity studies in rodents were presented without giving any short study summaries; in particular, the effects observed were not identified; (2) some of the key references are in Japanese with only the very brief abstract and some tables in English, including the key teratogenicity study in rabbits from which the notice selected the NOEL for the determination of the safe level; the English abstracts do not provide sufficient information for us to determine whether or not we have questions regarding the NOELs derived from these studies; (3) the summary of mutagenicity and genotoxicity studies were taken directly from the European Agency for the Evaluation of Medicinal Products (EMEA) Committee for Veterinary Medicinal Products (CVMP) summary report on 1-alpha-hydroxycholecalciferol (1998), which we consider to be opinions based on non-published studies. It is important to note that both publications in non-English text and non-published studies would not satisfy the general availability criteria for a GRAS determination.

9. The notice discussed safe medicinal use of 1-alpha-hydroxycholecalciferol in humans, and concluded that the thirty-year record of use, with a minimum of serious side effects, argues strongly that the recommended small doses (on the order of 1 μg or less) are safe in humans. However, people taking 1-alpha-hydroxycholecalciferol on a daily basis are recommended to test their blood calcium concentrations periodically due to concerns for hypercalcemia. We note that Gallagher *et al.* (1999) stated that the potential for toxicity (hypercalcemia and hypercalcuria) when using vitamin D analogs is minimal, if the recommended conventional doses are used (1

μg/day for 1-alpha-hydroxycholecalciferol), and, when appropriate doses are used, the incidence of hypercalcemia is less than 1% for this group of compounds. It is important to note that the evidence regarding the safety of human medicinal use does not usually constitute adequate evidence of the safety for human food uses. The clinical studies are inappropriate for the purpose of extrapolating the results to the general population; moreover, FDA's evaluation of a human drug considers both benefits and risks to patients, while FDA's human food safety evaluation of a feed substance, including substances considered to be GRAS, considers only potential risks in order to ensure a reasonable certainty of no harm to human consumers. From a human food safety perspective, we would have concerns about side effects that may not concern physicians administering a drug to humans. Therefore, we have questions about the appropriateness of extrapolating from a safe medicinal use (when risk and benefit to the human patient are considered) to a safe use in animals intended for human food.

10. The notice set a safe level of 1 μg/day based on a NOEL of 0.02 μg/kg bw/day from the teratogenicity study in rabbits and converted to *per* person *per* day using a 110-pound pregnant woman, without considering animal-to-human and human-to-human variability in responses (*i.e.*, there were no safety factor Products (EMEA, 1998) established an acceptable daily intake (ADI) of 0.002 μg/kg bw (0.12 μg/person) for 1-alpha-hydroxycholecalciferol on the basis of the NOEL of 0.2 μg/kg bw/day from the 3-month repeated-dose oral toxicity study in rats and a safety factor of 100.[1] The GRAS monograph has been previously reviewed by CVM, and concerns regarding lack of consensus among qualified experts about the safety of 1-alpha-hydroxycholecalciferol were raised, such as the existing opinions that there is insufficient evidence to define a safety threshold, including a lack of information on nonskeletal effects. There is clearly a lack of consensus in the establishment of a safe level.

11. The notice discussed human exposure due to consumption of edible tissues from chickens treated with the notified substance. We have questions regarding the assumptions made in the exposure estimates. The notice extrapolated the half-life in chickens based on studies conducted in rats and dogs. The notice also assumes an estimated 15-hour withdrawal period from treatment prior to slaughter (chickens are fasted at least 12 hours prior to transport to the slaughter facility, with another three hours prior to processing). We note that current VICH guidelines and our GFI #3 both consider practical zero withdrawal in chickens to be a maximum of six hours post treatment. The estimate also assumes that the exposure is primarily driven by the amount remaining in blood using half-life estimates from the non-target species, with an additional factor based on the assumption that 99.9% of Vitamin D metabolites are bound in blood. The single residue study provided from Covance Laboratories is unpublished and is missing several sections, resulting in questions regarding the in-life portion of the study, withdrawal period of the treated birds, and sample storage and processing. The study used an isotope labeled 25-hydroxy vitamin D3 for the internal standard. Because the report noted that the laboratory found that the two

[1] Though we note that the EMEA summary report (1998) showed that the NOEL established from the 3-month rat study is 0.02 μg/kg bw/day, thus the ADI would be 0.0002 μg/kg bw/day (or 0.012 μg/person) using the NOEL of 0.02 μg/kg/day.

compounds (25-hydroxy vitamin D3 and alphacalcidol) reacted differently during sample purification and instrumental analysis, this calls into question the appropriateness of the internal standard and the validity of the concentration values reported in the Covance study.

12. The notice stated that 1-alpha-hydroxycholecalciferol currently is fed to broilers throughout Latin America and Asia with no reports of adverse events. It is unclear whether they are referring to adverse events in chickens or human consumers. In addition, questions remain as to the number of years of such use in these regions, the quality of the adverse events reporting system in these regions, differences in susceptibility to toxicity of 1-alpha-hydroxycholecalciferol, consumption of chicken meats, and Vitamin D from other sources (sun exposure, fortified food and dietary supplement).

FDA has the following comments regarding target animal safety:

13. No other publications in the public domain were noted reporting the results of target animal safety for 1-alpha-hydroxycholecalciferol in broilers, except for a 2010 publication by Pesti and Shivaprasad. This publication reported the safety results of the 42-day, broiler safety study, which was sponsored by you. Data generated from this study are recognized as pivotal to your GRAS determination. CVM reviewed study documents of your study provided in February 2012 in response to our January 2012 request. In FDA's view, the target animal safety study that you rely on to establish safety lacks data that raise questions about the safety of 1-alpha-hydroxycholecalciferol when fed to broilers. Examples for those deficiencies of the study report and GRAS notice include that there were (1) no report on the findings of potential, compound-related medial degeneration in aorta of 1-alpha-hydroxycholecalciferol-treated broilers, (2) no sufficient investigation and report on the causality investigation of study early mortalities, and (3) no formulation and analyses on amount of 1-alpha-hydroxycholecalciferol in the feed. The above findings cause CVM to question your GRAS notice.

We have the following administrative recommendations regarding the notice:

14. The firm should number the pages of the notice consecutively throughout the document instead of independently numbering each of the sections.

15. The evaluation of a product label or labeling is not part of the GRAS notice evaluation process; however, the VDI could submit the label for a separate review independent of the GRAS notice, after a decision is made regarding the submitted notice.

16. A section dedicated to addressing the intended use of the proposed substance as a source of vitamin D_3 activity when added to broiler chicken feed at the rate of 5µg/kg feed is lacking in the submitted GRAS notice. The section of the GRAS notice titled "Occurrence and use" which contains statements and references about therapeutic use of the proposed substance in man and animals, and which only briefly mentions the current use of the substance in South America in broiler chicken feed should instead have addressed the intended use of the substance.

Conclusions

FDA has evaluated the data and information in AGRN 000-009 as well as other available information. The notice does not provide a sufficient basis for a determination that 1-alpha-hydroxycholecalciferol is GRAS under the conditions of its intended use in animal food.

In accordance with the Federal Register notice announcing the CVM Pilot Program, a copy of the text of this letter responding to AGRN 000-009, and a copy of the information in this notice that conforms to the information described in your GRAS exemption claim is available for public review and copying via the FDA home page at http://www.fda.gov. To view or obtain an electronic copy of this information, follow the hyperlinks from the "Animal & Veterinary" topic to the "Products" section to the "Animal Food & Feeds" to the "Generally Recognized as Safe (GRAS) Notifications" page where the Animal Food GRAS Inventory is listed.

If you have any questions about this letter, please contact Dr. Andrea Krause at 240-276-9768 or by email at andrea.krause@fda.hhs.gov. Please reference AGRN 000-009 in any future correspondence regarding this submission. If VDI wishes to have FDA consider any new information regarding 1-alpha-hydroxycholecalciferol, the appropriate mechanism would be for the notifier to submit, in accordance with proposed 21 CFR 570.36, a complete GRAS notice. FDA would assign a new file number to a new notice regarding 1-alpha-hydroxycholecalciferol.

Sincerely,

/s/
Sharon A. Benz, Ph.D., PAS
Director
Division of Animal Feeds
Center for Veterinary Medicine